Supplementary Fig. 2. Presence of a functional circadian clock in 3 distinct regions of the bladder. The detrusor smooth muscle (A, B), sphincter smooth muscle (C, D), and uroepithelial layer (E, F) of the bladders of adult Per2::Luc mice were dissected and examined regarding Per2 promoter-driven luciferase activity using a dissecting microscope. Clock gene expression was analyzed in the 3 bladder tissues, taken from mice under the AF and DF feeding schedules. Both AF and DF groups were acclimated to a 12:12 LD photoperiodic cycle for 1 week before being kept under DD conditions. Two days after the cessation of the LD cycle, the mice were sacrificed by cervical dislocation at a predetermined circadian time. Levels of Per1 (A, C, and E) and Rev-erbα (B, D, and F) mRNA were quantified using real-time polymerase chain reaction. All mRNA levels were normalized to the Tbp mRNA level and are presented as mean ± standard error of the mean (n = 4 per group). AF, ad libitum feeding; DF, daytime feeding; LD, light-dark; DD, constant darkness; mRNA, messenger RNA.